current and fluorescence signals, which corresponded to specific VSD rearrangements during channel activation. We found that the G406R mutation dramatically altered the operation of VSDs I and III compared to wild-type channels, by inducing a hyperpolarizing shift in their activation voltage dependence of ~80mV and ~50mV, respectively. These shifts were associated with a significant reduction in the effective valences of VSD I and III by ~50% and ~42%, respectively. Moreover, the sign of the fluorescence signals detected from TS channels was opposite to that observed in wild-type $Ca_V1.2$ channels. Taken together, these results suggest that the TS-causing mutation causes an overall structural perturbation, manifested as a change in both the fluorophore quenching process reported from VSD I and VSD III and their altered voltage-dependence. Funded by: NIH,AHA,FONDECYT,ACT.

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Three Splice Variants of the Calcium Channel Beta4 Subunit Display Differential Targeting and Gene Regulation in Neurons

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The β subunits of voltage-gated calcium channels regulate surface expression and gating properties of Ca_V1 and Ca_V2 α_1 subunits. All four $Ca_V\beta$ genes are expressed in the brain, but only mutation/lack of β_4 causes a neurological phenotype (epilepsy, ataxia) in humans and mice. The β_4 isoform is also targeted into the nucleus. There it directly interacts with the epigenetic machinery, suggesting a calcium channel-independent role of β_4 in transcriptional regulation. $Ca_V\beta$ subunits are subject to abundant alternative splicing. However, little is known about the specific functions of individual β splice variants in excitable cells. Here we identified a new alternatively spliced β_4 transcript, β_{4e}. mRNA and protein of this splice are highly expressed in mouse cerebellum and cultured cerebellar granule cells (CGC). Overexpression of β_{4e} modulates P/Q-type calcium currents in tsA cells and promotes surface expression of native synaptic $Ca_V2.1$ channels in hippocampal neurons. Compared to the other two known full-length β_4 variants (β_{4a}, β_{4b}) β_{4e} is most abundantly expressed in the distal axon. Consistent with the described role of N-terminal sequences in nuclear import, β_{4e}, which lacks these sequences, does not show nuclear targeting. The importance of nuclear targeting for the putative role of β_4 in transcriptional regulation was examined by whole genome expression profiling of CGCs from β_4-null mice individually reconstituted with β_{4a}, β_{4b}, or β_{4e}. Strikingly, the capacity of β_4 splice variants to regulate neuronal genes depended on their nuclear targeting with a rank order $\beta_{4b}>\beta_{4a}>\beta_{4e}$. Together these findings indicate that in neurons the three β_4 splice variants serve distinct functions. Whereas β_{4b} plays a dual role in channel modulation and gene regulation, the newly detected β_{4e} variant functions primarily as calcium channel subunit. *Support: FWF P23479, P24079, W1101, F4406.*

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Identification of a Determinant of High Affinity Calcium Binding in the Selectivity Filter of a Mammalian Calcium Channel

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Voltage-gated calcium channels (CaVs) provide the primary source of calcium influx in excitable cells and couple electrical signals to chemical signaling cascades. Due to CaV size and the difficulty of expressing CaVs at quantities sufficient for high-resolution determination, detailed structural information is limited to isolated cytoplasmic domains. However, CaVs are homologous to voltage-gated sodium channels (NaVs) and NaV structure can serve as a template for CaV structure. We determined the structure of the closed conformation of NaVAe1p, a pore only bacterial NaV derived from NaVAe1, an Alkalilimnicola ehrlichei bacterial NaV. This structure reveals the site of a putative calcium ion at the extracellular mouth of the selectivity filter liganded by four serines. At the equivalent site in mammalian calcium channel selectivity filters, there is a conserved aspartate in one of the calcium channel domains. Our functional studies show that this aspartate is a previously unknown determinant of CaV high affinity calcium binding in the mammalian calcium channel CaV1.2. These findings show the extent of similarities between bacterial sodium channels and eukaryotic voltage gated channels and shed new light on the selectivity filter in mammalian calcium channels.

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Functional Clustering of L-Type CaV1.3 Channels

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CaV1.3 channels play a critical role in pace-making of sinoatrial node cells and in the repetitive firing of neurons. We investigated the function and spatial organization of CaV1.3 channels in tsA-201 cells and in human iPSC-derived cardiomyocytes (hiPSC-CM) using patch-clamp, TIRF and super resolution GSD microscopy. The activation of CaV1.3 channels in these cells produced local Ca^{2+} signals called "CaV1.3 sparklets". Sparklet activity varied regionally with some regions of the surface membrane showing higher activity than others. The amplitude of the elementary CaV1.3 sparklet was about 40 nM. Sparklets with discrete amplitudes of 80, 120 and 160 nM were frequently observed, suggesting the simultaneous opening of 1-4 CaV1.3 channels. We used GSD super resolution imaging, with a spatial resolution of approximately 30 nm, to determine the organization of CaV1.3 channels. As shown in the super resolution map in Fig. 1, we found that CaV1.3 channels were expressed in clusters, of variable sizes and geometry, distributed at irregular intervals throughout the cell membrane. Our data suggest that CaV1.3 channels organize in clusters and that the simultaneous activation of channels within these clusters can produce high-amplitude Ca^{2+} signals.

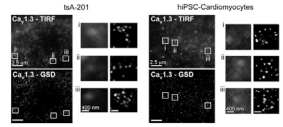

Fig. 1. GSD super-resolution images of CaV1.3 channels in tsA-201 cells expressing the channels and in hiPSC-derived cardiomyocytes.

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Calcium Channel $\alpha_2\delta$-1 Subunit Knockout Causes Diabetes Due to Impaired Insulin Release

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Pancreatic β-cells, the major cellular component of the island of Langerhans, are responsible for the synthesis of insulin and its secretion in response to elevated blood glucose. High voltage-gated calcium channels (HVCC) are intimately involved in excitation-secretion coupling in pancreatic islet cells and calcium entering through HVCC is an important regulator of insulin synthesis. HVCC are multi-subunit protein complexes comprised of the main pore-forming α_1 subunit and auxiliary extracellular $\alpha_2\delta$ and intracellular β subunits. Here we show that genetic ablation of the $\alpha_2\delta$-1 subunit (the main pancreatic $\alpha_2\delta$ isoform) results in the postnatal development of diabetes. Homozygous $\alpha_2\delta$-1 KO mice show highly elevated urine production and develop ~9-fold higher blood glucose levels compared to WT littermates. Morphological analysis of the pancreas shows a reduction in the number of islets and their size due to a dramatic decrease in β-cell mass in an age-dependant manner. The reduced β-cell mass is not caused by an islet-specific autoimmune reaction, but might result from prolonged hyperglycaemia toxicity. Voltage-clamp recording in dissociated pancreatic β-cells shows a more than two-fold decrease in calcium current amplitude. Glucose stimulated calcium oscillations in whole isolated pancreatic islets shows a strong decrease in amplitude of both the first and second phase of insulin release, and an increased oscillation frequency of the second phase. On-going pharmacological experiments will identify which pore-forming α_1 subunits are primarily affected by $\alpha_2\delta$-1 deletion and how this effects insulin secretion. These findings indicate that $\alpha_2\delta$-1 is an important determinant of normal β-cell physiology, critical for insulin release. Support: FWF W1101, P23479, LFU-P7400-027-011.

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Chemical-Biological Generator of Step Increases in Calmodulin Reveals Dual Modulation of L-Type Ca^{2+} Channels

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Voltage-gated L-type Ca^{2+} channels are prominent Ca^{2+} entry portals into many cells. Proper functioning of these channels requires Ca^{2+}-dependent inhibition (CDI) of channel opening by calmodulin (CaM). CDI requires that